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A Papillary Cystic Disease Affecting the Barbels of *Ameiurus nebulosus* (Le Sueur), Caused by the Myxosporidian *Henneguya ameiurensis* sp. nov.

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(Plates I-VIII; Text-figure 1).

Introduction.

Many of the myxosporidians are histozoic parasites of fishes and cause only slight host tissue responses. Occasionally, lesions are produced that eventually develop into large tumor masses (see Nigrelli & Smith, 1938). The tissue response in such cases manifests itself by the proliferation of fibroblastic material which forms a supportive frame-work for the developing spore masses. The end result in such growths, however, is the degeneration of muscle, bone and other tissues in the path of the growing fibroblasts and spores. It is interesting to note that in many of these myxosporidian infections the inflammatory reaction is invariably mild and only when a secondary bacterial or fungal infection sets in does it become severe, the latter occurring perhaps at the time the tumors rupture to release the mature spores.

As a general rule, histozoic myxosporidians are definitely localized by circumscribing connective tissue. Their effects on host tissues are usually limited to these circumscribed areas and only in certain instances do they cause pathological changes to adjacent tissues and other organs. The present contribution deals with an instance where not only a local phenomenon occurs, but also immediate neighboring tissue responses develop. In the disease to be described here, the myxosporidian infection is localized in the connective tissue layer of the skin of the barbels of the common bullhead. Although the parasites are encapsulated by host tissue and although they elicit degenerative changes within these enclosed areas, nevertheless they also induce pathological changes adjacent to the encapsulated collections of organisms, such as hyperplasia of the overlying epithelium. The details of these two sets of changes will be described below.

The parasites causing this papillary cystic disease belong to the genus *Henneguya* Thélohan. Surprising as it may seem, it is the first myxosporidian species to be described from the common bullhead, *Ameiurus nebulosus* (Le Sueur). This parasite, however, differs sufficiently from other species of *Henneguya* to be considered new and for which the name *Henneguya ameiurensis* is proposed.

Material and Methods.

The infected catfish was taken from a lake in New Hampshire during the summer of 1938. The disease was found in a single fish and attempts to obtain more material were without success, although a great many fish were examined for this purpose.

The fish was an adult, measuring 7½ inches in total length. As seen in the accompanying drawing (Fig. 1) and photograph (Fig. 2), the disease affected the barbels, especially the two dorsal ones, and the barbel at the angle of the mouth on the right side of the body. Externally there were no other manifestations of the disease and the fish appeared normal in all other respects.

The bases of the affected barbels were completely surrounded by tumor masses (Figs. 1, 2). On closer inspection these masses had a very distinct, irregular papillary appearance. The parts of the barbels distal to the lesions, however, were normal in appearance.

The material was fixed in 10% formalin and numerous blocks were prepared for histological studies. They were sectioned at 5-10 microns and stained with hematoxylin-eosin, Giemsa's, Mallory's and Masson's.

Description of ***Henneguya ameiurensis*** sp. nov.

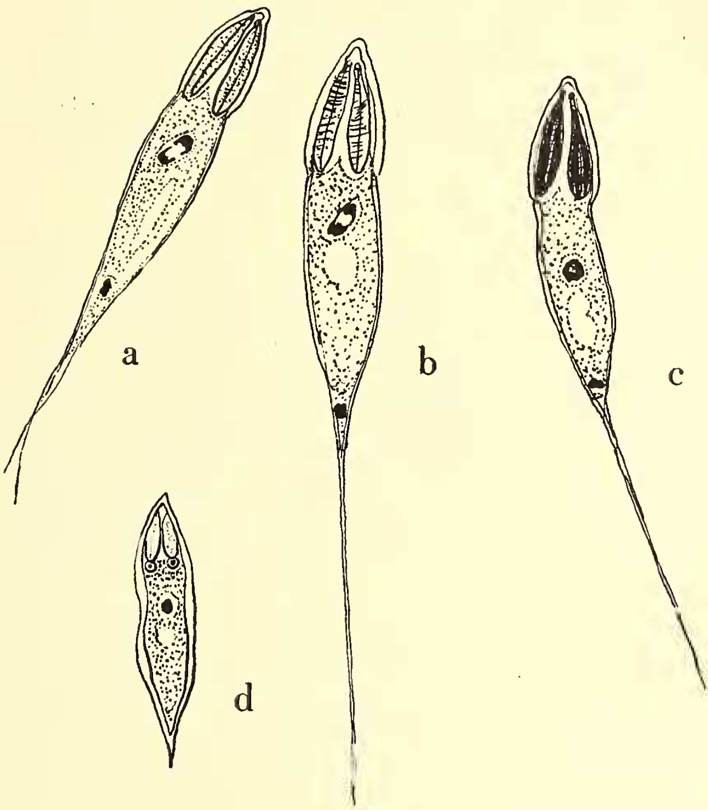
Vegetative Stages: The cysts containing the masses of organisms were found in the dermal connective tissue of the barbels (Figs. 3, 4, 5). The cysts varied considerably as to shape and in size. In certain cross sections of the barbels as many as fifteen cysts were found, of which a few were spherical but the majority of which were oval or irregular in shape. They measured from .190 x .342 to .760 x 1.22 mm. In practically all instances, the cysts were matured, containing for the most part fully developed spores. However, pansporoblasts were present at the periphery of the cysts, but in the majority of the cases they were fairly well advanced in their development. In younger cysts all stages of sporogenesis were recognized (Figs. 6, 7). The pansporoblasts are disporous, although monosporous forms were evident. *Henneguya ameiurensis* is polysporous.

The young sporonts are spindle-shaped organisms. Polar capsule nuclei are present and the sporoplasm contains but a single nucleus (Text-fig. 1, d). As the sporont becomes mature, the definite capsules are completely developed and the shell with the posterior process begins to elongate (Figs. 6, 7). The two nuclei found in the adult spore appear to be the result of a subsequent division, for many spores were found with dividing nuclei in the telophase stage (Text-fig. 1, a, b). According to Kudo (1926), the sporont of many Myxsosporidia contains six nuclei, two of which form the capsule, two for the shell and two remain as the nuclei of the sporoplasm.

It is interesting to report here that many uninucleate, irregular shaped organisms were encountered within the cysts, scattered among the matured spores. These have been interpreted by us as newly released sporoplasms which will perhaps migrate from the cysts into adjacent tissues and set up new foci of infections. Such possible migrations might also explain certain connective and epithelial reactions encountered in these preparations.

Spores: The spores are more or less lanceolate in shape, being approximately six times as long as they are broad (Text-fig. 1; Figs. 8, 9, 10, 11). The mature forms average about 23.3 microns in length, 4.1 microns in width, 3.0 microns in thickness. The posterior process varies considerably in length and thickness. The more elongated the process, the thinner it is. However, the length measurements for this process vary from 15-41.5 microns. The process is an extension of the shell material and therefore composed of two halves. Occasionally they may be separated along the posterior tip, giving it a bifurcated appearance (Text-fig. 1a).

The sporoplasm is granular and in the majority of forms binucleated. Uninucleate stages were rare in our material. A small iodinophilous vacuole was occasionally found anterior to the nuclei but most always it was found posterior to them. At the posterior end of the sporoplasm, at about the point where the posterior process begins, one or two deeply staining basophilic granules are always present, (Text-fig. 1; Fig. 11). The nature of these granules is unknown, although they have been seen and described by other investigators (e. g., Gurley, 1888).



Text-fig. 1.

Henneguya ameiurensis sp. nov. from the barbels of the common bullhead, *Ameiurus nebulosus*. **a**, **b**, **c**, mature spores. Note dividing nuclei in a and b. **c**, uninucleate spore. **d**, young spore with uninucleate sporoplasm and two polar capsule nuclei. × 1500.

Although the general shape of the shell is lanceolate, there is a characteristic indentation at the level of the posterior end of the polar capsules (Text-fig. 1; Fig. 10). The sutural ridge is faintly evident. The polar capsules measure on the average about 5.4 microns in length and 1.6 microns in width at their greatest diameter. They do not, however, fill the entire anterior end of the shell. The anterior end of the shell tapers slightly at the tip, giving it a blunt conical appearance.

The staining reactions of the spores to the various techniques used may be mentioned here. With Masson's stain the shell, capsules and vacuole are colored a very dark green; polar filaments and sporoplasm take on a red color; while the nuclei are stained a deeper red. With Mallory's stain the shell and capsules are colored yellowish; sporoplasm is orange; nuclei and posterior sporoplasmic granules take on a light blue color. With Giemsa's stain the polar filaments take on a deep blue-green color; shell and extension are a lighter blue-green color; sporoplasm and vacuole take on a pinkish color.

In several of the cysts, spores with three and four polar filaments and nuclei were encountered. Similar spore abnormalities were described by Schäferna & Jírovec (1931) for *Henneguya acerinae.*

Comparisons: *Henneguya ameiurensis* appears to be closely related to *H. gurleyi* Kudo (1910 (=*Myxobolus* cf. *linearis* Gurley, 1894), a species found in lesions at the base of the spines of the second dorsal fin of *Ameiurus melas.* However, *H. ameiurensis* differs from *H. gurleyi* in the shape of the shell and in size measurements, two important diagnostic characters. *Ameiurus nebulosus,* the common bullhead, insofar as is known to the authors, is a new host species for which a myxosporidian infection has been reported.

Description of the Myxosporidian Lesion in *Ameiurus nebulosus.*

The cross section of the barbel, including the tumor, reveals the fact that the tumor mass arises from the skin at the base of the barbel (Figs. 3, 4). Part of the tumor is composed of numerous cystic cavities separated by connective tissue septa (Fig. 5). These cavities contain the organisms referred to above. Each cyst is surrounded by a dense fibrous capsule varying in thickness from 6 to 16 microns. The septum separating two adjacent cysts is formed also from fibrous tissue varying somewhat in density. This enclosing fibrous tissue is composed of fusiform connective tissue cells, in places hyalin, with here and there a few scattered lymphocytes. The inner lining of the cysts is formed of very flattened fibroblastic cells. In contact with the inner lining may be found globular or slightly flattened masses which are the developing schizonts of *Henneguya ameiurensis* (Figs. 5, 6, 7). The remainder of each cavity is loosely packed with large numbers of young and matured spores.

It is obvious that the cysts occupy the region directly below the epithelium; that is, they lie in the corium or just below this layer.

All of the cysts seem to contain varying amounts of a stringy mucoid substance in which may be found embedded small numbers of spores. Other cysts contain, beside the mucoid material, lymphocytes, some fibroblasts and occasional melanophores (Fig. 12). The presence of these cellular elements invading the cavity may indicate an early healing response. Later stages of repair were not encountered in our material. It would be interesting to know the final fate of the cysts and their contents, for it might shed light on the subsequent stages of the developmental cycle of these organisms. Our recent studies on lymphocystic disease in its later stages has indicated the probable fate of these large hypertrophied cells characteristic of the disease by a rupture of the hypertrophied lymphocystis cell, permitting the escape of their contents into the surrounding water (Nigrelli & Smith, 1939). Here, again, the same outcome may be effected, particularly as we find in greatly distended cysts occasional areas of thin overlying epithelium.

As stated earlier in this paper, the finding of released sporoplasms may indicate that reinfection of adjacent tissues occurs when these organisms migrate from the cysts, without actual rupture of the cyst wall.

As a rule, however, the epithelium is considerably thickened over the infected area, giving the lesion a papillary or warty appearance. The epithelium is composed of many layers and contains mucus cells and giant dermal gland cells, the latter referred to in German literature as "Kolben Zellen" (Figs. 13, 14). Reed (1924) has called these last-named giant cells dermal gland cells and according to him they arise from elements which he termed "clavate" cells. Dermal gland cells are demonstrated very clearly in our material, as can be seen in Fig. 15. They are often binucleate and stain a faint pink or yellow color with eosin and Masson's respectively. Their nuclei are intensely basophilic. Such cells seem to be plentiful in the normal skin of the catfish barbel. In the infected barbel, however, they appear to be increased in numbers, just as are the mucus and squamous cells of the overlying skin. Their exact function is not known.

The sensory end organs are retained in the hyperplastic skin overlying the tumors without evidence of degeneration. They probably are not increased in numbers (Fig. 16).

Melanophores are of very frequent occurrence in the papillary epithelium and in places suggest a definite hyperplasia of this type of cell (Fig. 17).

Histological examination of sections of other organs and tissues of the catfish showed their structure apparently normal in all respects.

Discussion.

Tissue responses to myxosporidian infections have been reported by other investigators (Gurley, 1893; Hahn, 1915, Kudo, 1926, 1929; Bond, 1938; etc.). Kudo (1926, 1929) has noted and described the histo-pathology in lesions due to Myxosporidia, and in 1929 he reported the development of a hyperplastic growth of the epithelium of the gills in channel catfish, *Ictalurus punctatus*, infected with *Henneguya exilis*. In these catfish, the parasites are encysted on the gill lamellae, and the surrounding tissue responded to the infection by an enormous hyperplastic growth of the adjacent epithelium together with a severe inflammatory reaction. In the present case, the inflammatory reaction is a mild one but there is a definite hyperplasia of the epithelium overlying the subcutaneous infected regions, and such specialized cells as melanophores, mucus and dermal gland cells found in the epithelium are involved to a certain extent in this hyperplasia.

Summary.

1. A papillary cystic disease is described affecting the barbels of *Ameiurus nebulosus* (Le Sueur).

2. The parasite producing this disease is a new species of *Henneguya* Thélohan, for which the name *H. ameiurensis* is proposed.

3. The spores are more or less lanceolate in shape, measuring 23.3 x 4.1 x 3.0 microns. The posterior shell extension, characteristic of the genus, varies considerably in length and thickness.

4. The binucleate condition of the mature sporoplasm appears to be the result of division of the nucleus in a slightly earlier stage of the spore.

5. The pansporoblasts are disporous, although monosporous forms do occur. This myxosporidian species is polysporous.

6. *H. ameiurensis* is the first myxosporidian parasite to be described from the common bullhead.

7. The various tissue responses of the host to this form of myxosporidian infection is described.

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EXPLANATION OF THE PLATES.

PLATE I.

Fig. 1. Drawing of a formalin-fixed specimen of *Ameiurus nebulosus*, showing papillary cystic disease at the bases of the dorsal barbels. Drawing by Clare Smith.

Fig. 2. Photograph of a front view of the catfish, demonstrating the papillary cysts of the dorsal barbels and the barbel at the angle of the mouth on the right side.

Fig. 3. Photomicrograph showing distribution, number, size and shape of the myxosporidian cysts in a cross-section of one of the catfish barbels. × 50. H-E.

PLATE II.

Fig. 4. Papillary cystic tumor caused by *Henneguya ameiurensis* arising from the base of a dorsal barbel. × 16. H-E.

Fig. 5. Higher magnification of section shown in Fig. 3. The organisms fill the entire cavity of each cyst. Note the vegetative masses at the periphery of the cysts. Note also the intercystic septa. × 125. H-E.

PLATE III.

Fig. 6. Photomicrograph showing the vegetative masses of *Henneguya ameiurensis* at the periphery of the cyst. Pansporoblasts, sporonts and young spores can be recognized. × 350. H-E.

Fig. 7. Photomicrograph of a slightly later stage than that shown in Fig. 6. Here sporogenesis is almost completed. × 500. H-E.

PLATE IV.

Fig. 8. Photomicrograph of young spores. × 400. H-E.

Fig. 9. Fully developed spores of *Henneguya ameiurensis*. Posterior extensions of the valves not in focus. × 1400. H-E.

PLATE V.

Fig. 10. Higher magnification of the spores. Note outline of the shell. × 1700. H-E.

Fig. 11. Mature spores. Spore in upper right-hand corner shows split posterior process and posterior basophilic granule. × 1500. H-E.

PLATE VI.

Fig. 12. Photomicrograph showing cysts with stringy mucoid material, lymphocytes, fibroblasts and isolated spores. Perhaps an early stage in healing giant dermal gland cells × 125. H-E.

Fig. 13. Photomicrograph showing the host tissue responses. Note the thickened epithelium, composed of many layers of epithelial cells, mucus cells and giant dermal gland cells. × 125. H-E.

PLATE VII.

Fig. 14. Higher magnification of the thickened epithelium shown in Fig. 13. × 350.

Fig. 15. Giant dermal gland cells found in the skin of the barbel of the catfish. A slight hyperplasia is to be noted for these cells in the infected barbel. × 400. H-E.

Plate VIII.

Fig. 16. Sensory end-organ in the hyperplastic skin overlying the myxospordian tumors. No degenerative changes are to be noted for these structures. × 350. H-E.

Fig. 17. Melanophores in the skin of infected portion of the barbels. The frequent occurrences of these cells in the papillary epithelium suggests a definite hyperplasia of this type of cell. × 275. H-E.

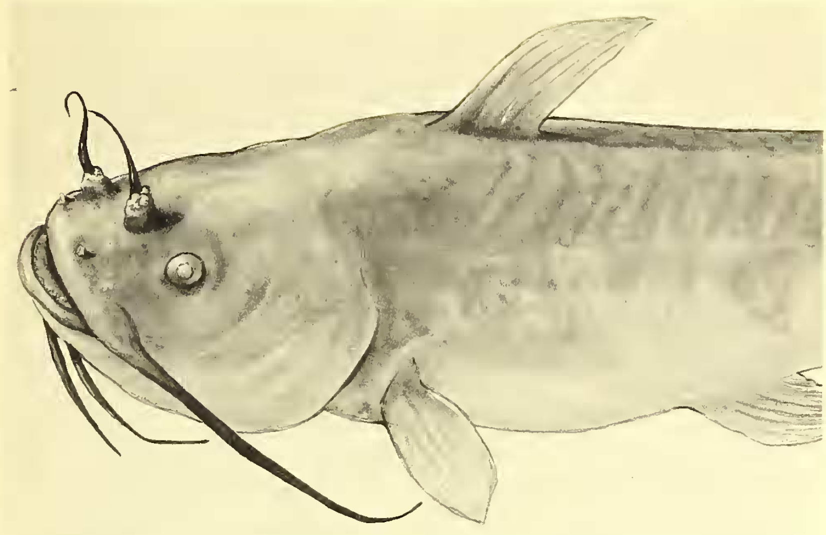


FIG. 1.

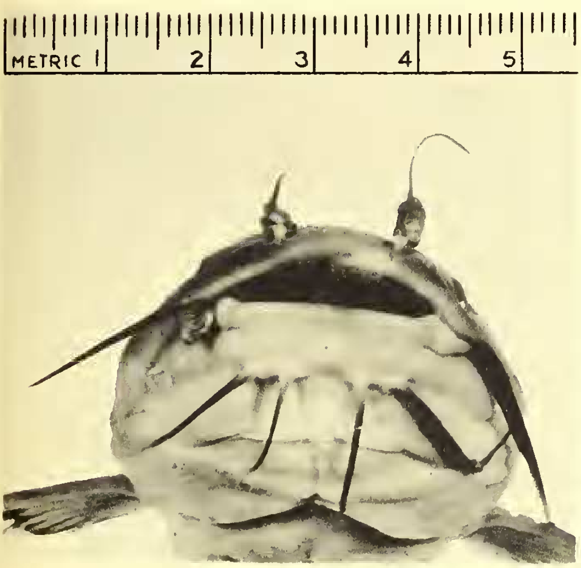


FIG. 2.

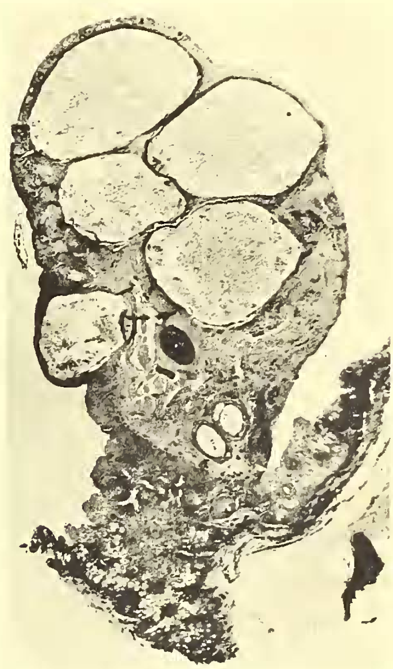
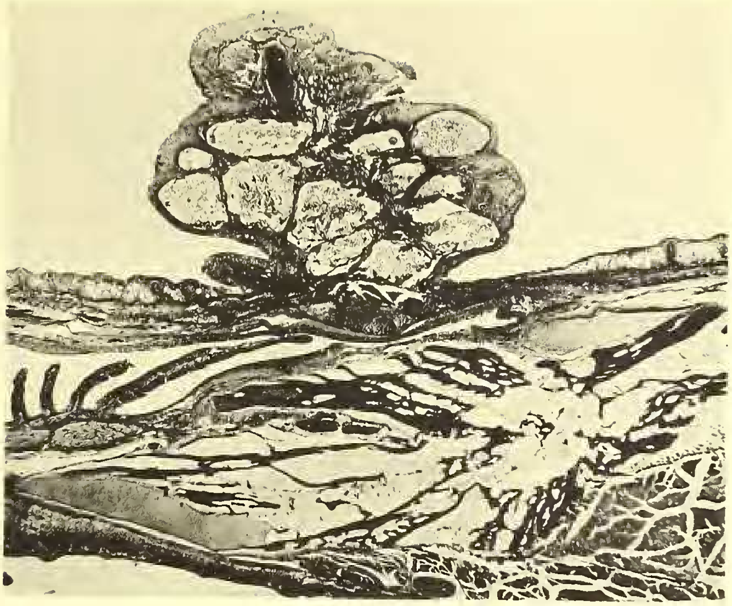


FIG. 3.

A PAPILLARY CYSTIC DISEASE AFFECTING THE BARBELS OF AMEIURUS NEBULOSUS (LE SUEUR), CAUSED BY THE MYXOSPORIDIAN HENNEGUYA AMEIURENSIS SP. NOV.



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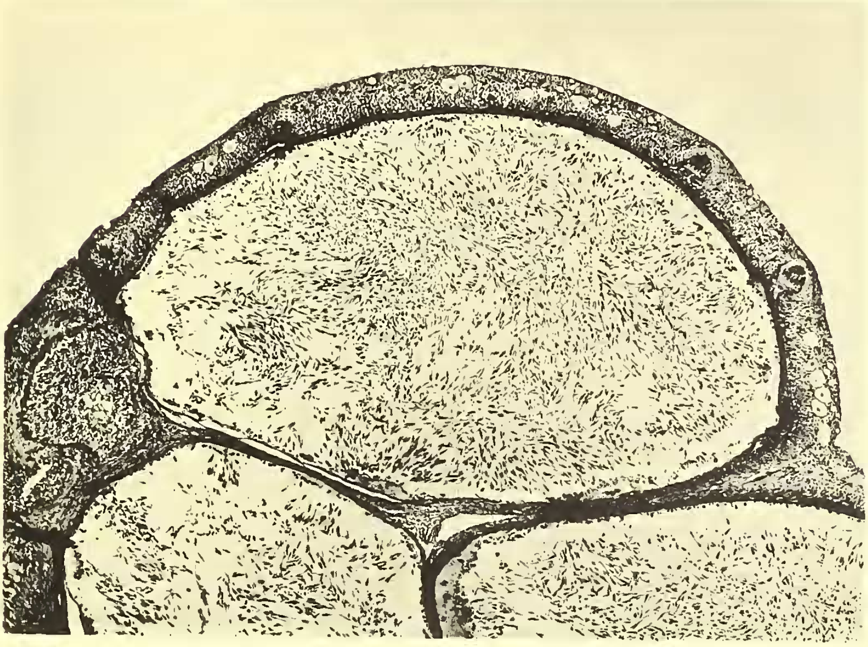

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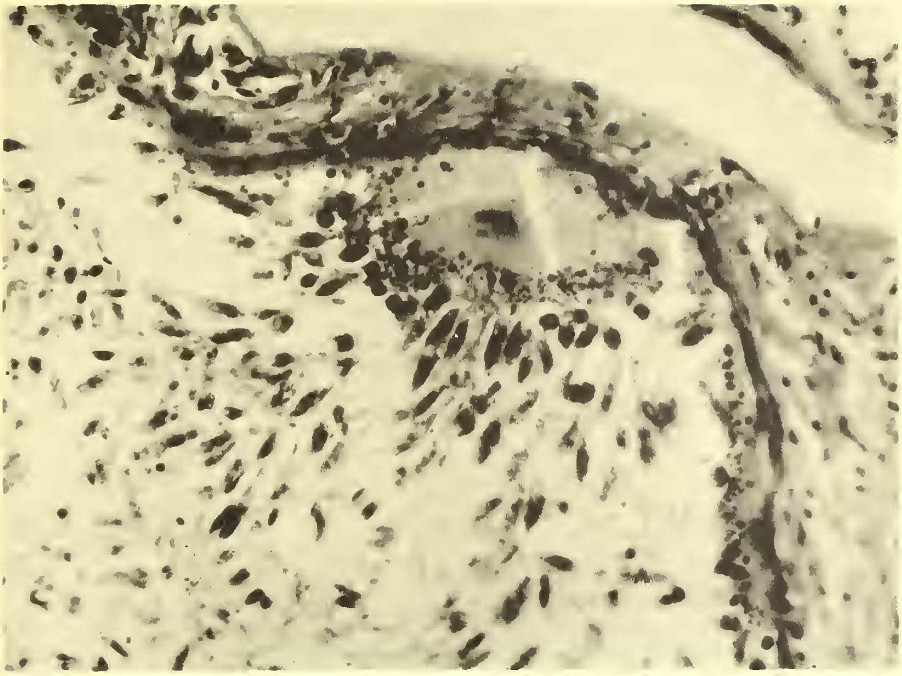
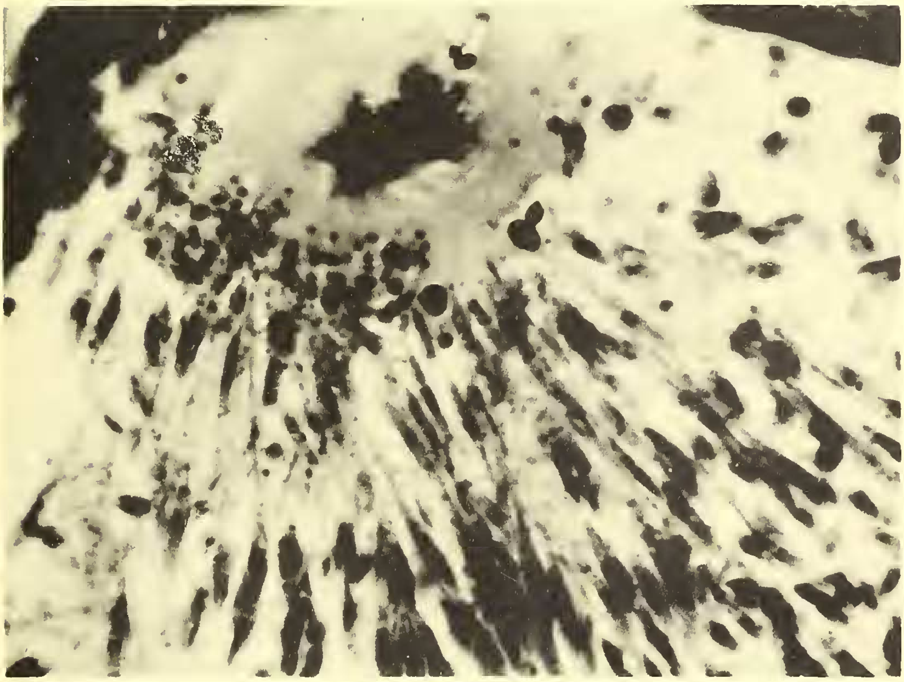


FIG. 6.



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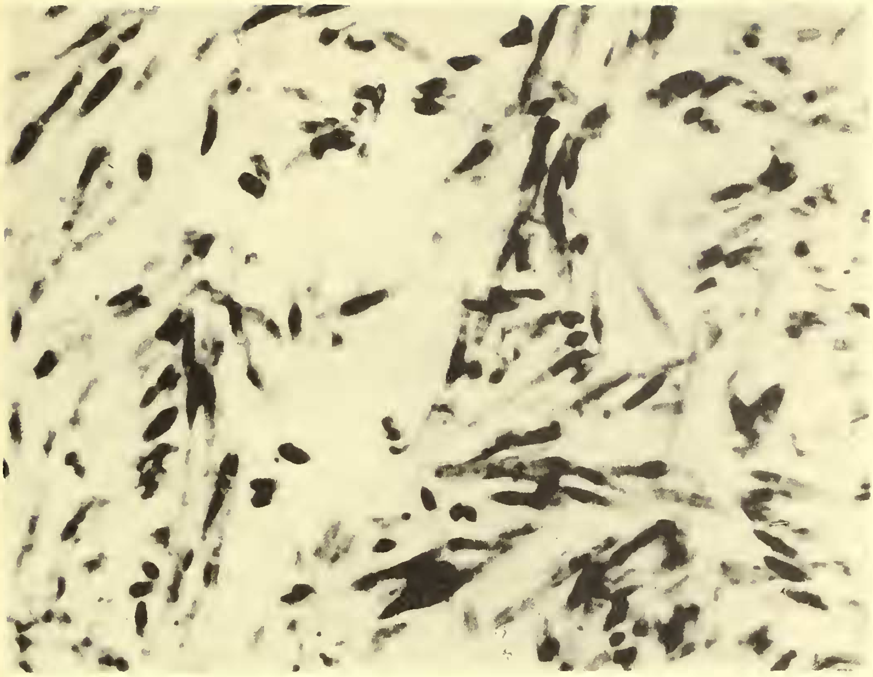

FIG. 8.

FIG. 9.

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FIG. 10.

FIG. 11.

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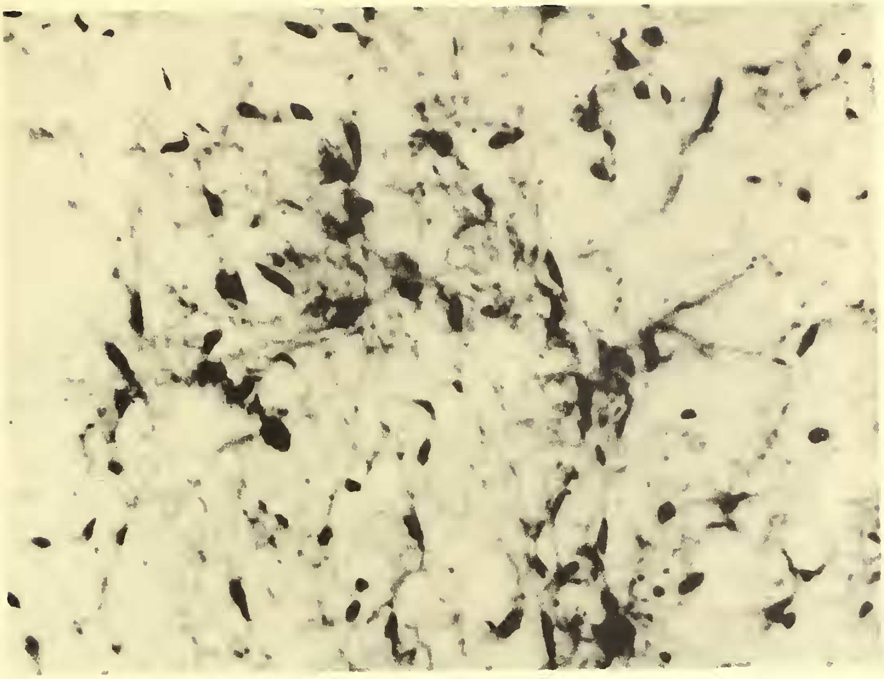
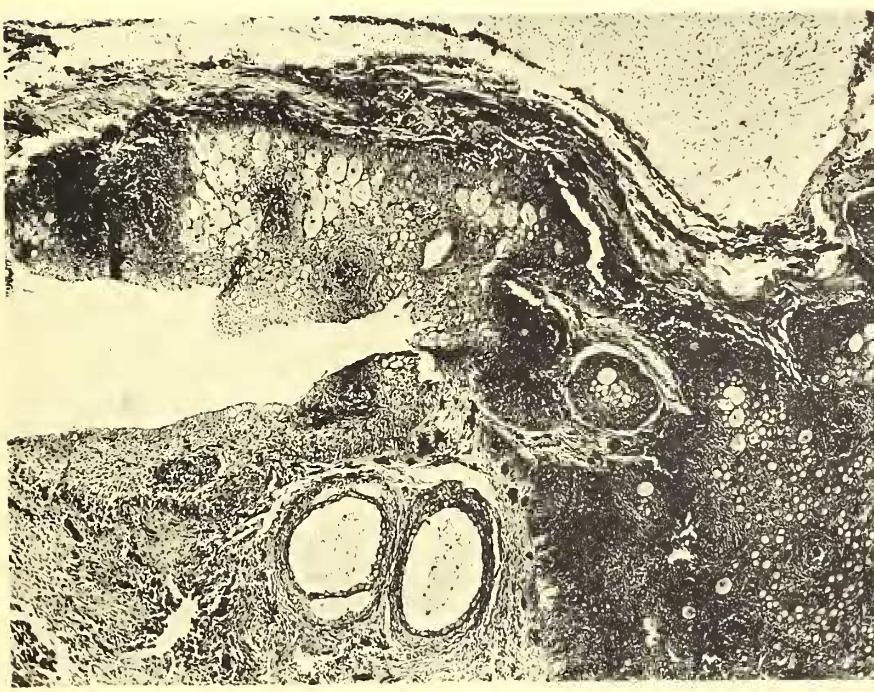


FIG. 12.



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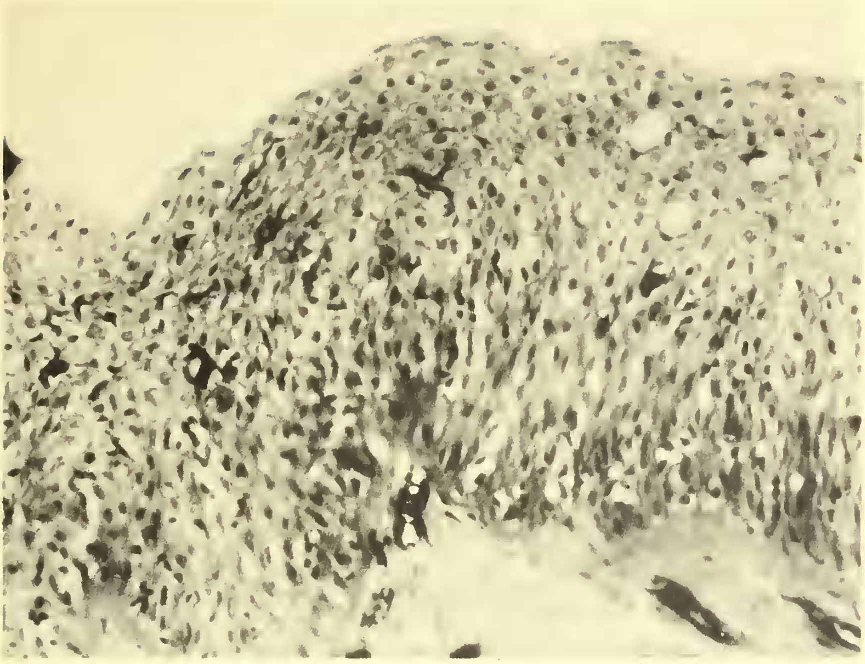

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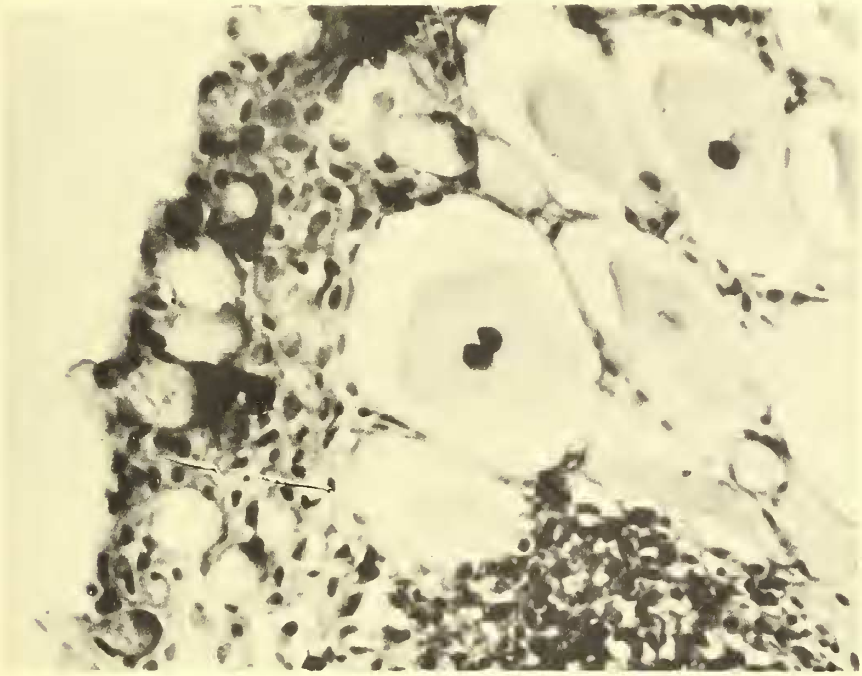

FIG. 15.

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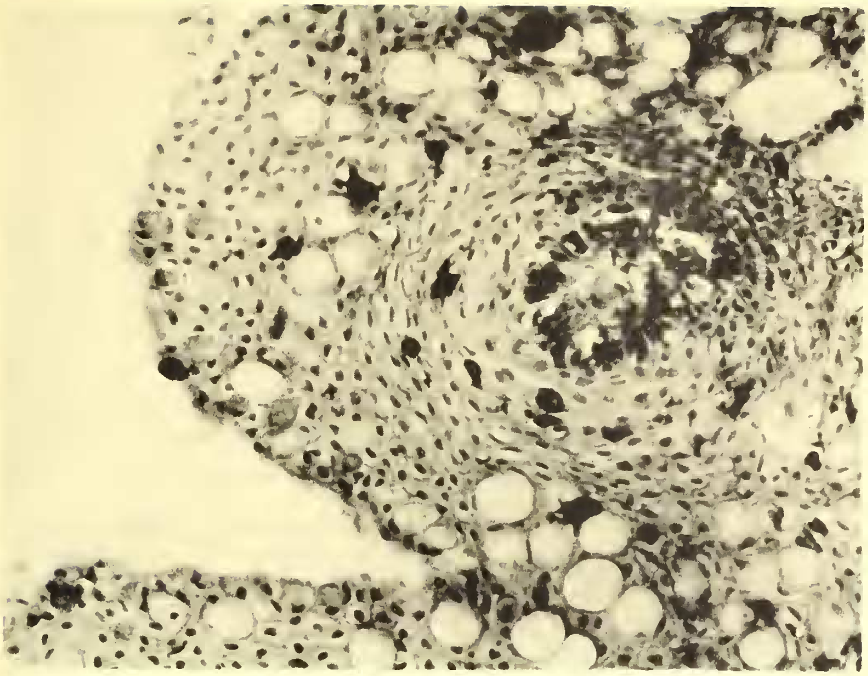

FIG. 16.

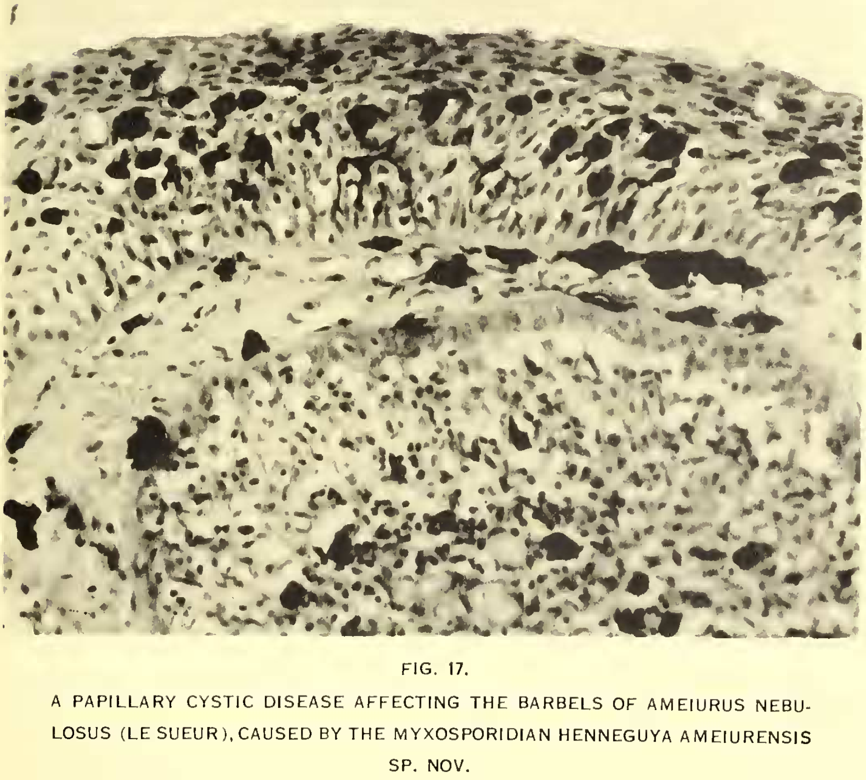

FIG. 17.

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